Reprinted from *J. Food Sci.*
46(6):1814-1816

A Re-Evaluation of the Products of Gamma Irradiation of Beef Ferrimyoglobin

KEVIN D. WHITBURN, MORTON Z. HOFFMAN, and IRWIN A. TAUB

ABSTRACT

Quantitative analysis of the interactions of ·OH and e_{aq}^- with ferrimyoglobin in neutral aqueous solution provides a basis for evaluating earlier studies of the radiolysis of myoglobin. γ-radiolysis of deaerated solutions in which either ·OH or e_{aq}^- predominates leads to the formation of both deoxymyoglobin and ferrimyoglobin peroxide. Correction for a post-irradiation thermal process allows radiation-induced composition changes to be separately investigated. Depending on the radical system under consideration, a specific steady-state composition is reached at moderate doses that involves the ferri, deoxy, and ferri-peroxide forms of the myoglobin. Aeration of irradiated solutions quantitatively converts the deoxymyoglobin to oxymyoglobin. Radiolysis of oxygenated solutions containing N_2O generates only ferrimyoglobin peroxide. Earlier studies on the radiolysis of ferrimyoglobin have been re-evaluated in light of novel features that have emerged in this investigation.

INTRODUCTION

GAMMA-IRRADIATION has been investigated widely as a means of sterilizing or pasteurizing meat products. Upon irradiation, the color pigment myoglobin is known to undergo chemical changes that result in discoloration of the meat sample. Previous qualitative studies spanning the last 25 years on the radiation chemistry of meat extracts and myoglobin solutions offer reasonable consensus that ferrimyoglobin is radiolytically reduced in aqueous solution. While Tappel (1956) believed that the red product in aerated solution is oxymyoglobin, others, including Bernofsky et al. (1959), Brown and Akoyunoglou (1964), Clarke and Richards (1971), and Satterlee et al. (1972), have suggested it to be a spectrally similar material. Ferrimyoglobin peroxide, which has absorption maxima similar to those of the oxy form (MbO_2) in the visible region, has been conclusively shown as a product component in the radiolysis of ferrimyoglobin (ferriMb) in aqueous solution by Bernofsky et al. (1959). This important product form, overlooked by other workers in the field, was confirmed by Giddings and Markakis (1972).

The product spectrum obtained by Bernofsky et al. (1959) under dose conditions high enough to ensure that no further spectral change occurred, was not that of MbO_2 or ferri-peroxide, but rather, was similar to both. These authors consequently concluded that the product was a modified oxygenated form of myoglobin of an "intermediate type." Addressing the effect of aeration on the product distribution in the radiolysis of ferriMb, Giddings and Markakis (1972) proposed that MbO_2 and ferri-peroxide were separately generated in deaerated and aerated solutions, respectively.

As part of our research on the radiation preservation of meat (Shieh et al., 1979), we have quantitatively studied the interaction of ·OH and e_{aq}^- radicals with myoglobin. From analyses of compositional changes induced by γ-radiation, we have been able to clarify the issues raised in these previous reports.

Authors Whitburn and Hoffman are affiliated with the Dept. of Chemistry, Boston Univ., Boston, MA 02215. Author Taub is affiliated with the Food Engineering Laboratory, US Army Natick Research & Development Laboratories, Natick, MA 01760.

EXPERIMENTAL

THE METHOD for isolation and purification of bovine muscle oxymyoglobin followed the techniques of Hardman et al. (1966) and Yamazaki et al. (1964). FerriMb was produced by oxidation of pure MbO_2 by $Fe(CN)_6^{3-}$ and removed by dialysis and chromatography. The deoxy (Mb) and ferri-peroxide dorms were generated from the ferri derivative by addition of excess $S_2O_4^{2-}$ and H_2O_2, respectively. The ferri-peroxide form has been rigorously studied by Fox et al. (1974); it may be formally designated as Mb(IV) (King and Winfield, 1963) in order to emphasize that it is one-equivalent higher than the oxidation state in ferriMb. Bernofsky et al. (1959) and Gidding and Markakis (1972) have used the older nomenclature of "ferrylmyoglobin" in reference to this material. We will use the symbol Mb(IV) to denote this material. The absorption spectra of the oxy, ferri, deoxy, and ferri-peroxide derivatives were accurately determined in the 450–700 nm region with ε-values based on $\epsilon_{580}(MbO_2) = 1.46 \times 10^4\ M^{-1}\ cm^{-1}$ (Hardman et al., 1966).

All solutions, buffered with phospate (0.01M) at pH 7.3, were thoroughly deaerated by saturation with N_2O or Ar. Irradiations were performed at 20 ± 2°C using γ-sources with dose-rates of 6.1 and 14.9 krad min^{-1}. Data collection consisted of multiple spectrophotometric scans in the 450–700 nm region before and after radiolysis.

Upon radiolysis, e_{aq}^- (2.8), ·OH (2.8), H_2O_2 (0.7) and H· (0.6) are generated in aqueous solution with the molecular yields per 100 eV of absorbed energy, or *G*-values, shown in parentheses. In N_2O-saturated solution, e_{aq}^- is rapidly converted to ·OH radical (Anbar et al., 1972). The reactive ·OH radical can be effectively removed by scavenging with *tert*-butyl alcohol, which in Ar-purged solution leaves e_{aq}^- as the dominant reactive radical.

RESULTS & DISCUSSION

THE SPECTRAL CHANGES incuded upon irradiation of ferriMb in deaerated aqueous solution show a depletion of the characteristic ferri peaks at 505 and 630 nm, accompanied by absorbance growth in the 520–610 nm region, for both ·OH and e_{aq}^- as precursor radicals. After irradiation there is a slow thermal regeneration of ferriMb absorbance at the expense of the absorbance growth, which was followed for a period of 0·5 – 1 hr. This thermal process has been reported at room temperature by Bernofsky et al. (1959). The observed spectral changes were analyzed in terms of the percent composition of the ferri, deoxy, and ferri-peroxide forms of myoglobin, based on the known ε-values for each prosthetic form and absorption measurements at three chosen wavelengths (500, 560, 590 nm) through the solution of simultaneous equations based on Beer's Law. The derived constancy of total [myoglobin] throughout each analysis is consistent with the absence of any significant quantities of absorbing species in solution other than those analyzed.

The spectral-based composition analysis shows that the radiolysis of deaerated solutions induces a conversion of ferriMb to *both* Mb and Mb(IV) and that the thermal regeneration of the ferri form is at the expense of both products. In order to examine the *radiation-induced* composition changes, component compositions were extrapolated through the early portion of the thermal process (through < 15% of the total thermal change) back to zero time, chosen as the mid-point of the radiolysis duration.

These analyses were firstly applied to N_2O-saturated solutions containing 30 ± 2 μM ferriMb γ-radiolyzed with doses of 3.1–55.2 krad. The γ-induced composition changes, corrected for the post-irradiation process, are shown as a function of dose in Figure 1a. For doses above ∿30 krad, the solution composition is essentially independent of dose for further moderate increases, remaining 44% ferri, 18% deoxy, and 38% ferri-peroxide. Solutions initially 34% or 100% Mb(IV) approach the same steady-state, but at significantly higher doses. The G(Mb) and G(Mb(IV)) values are plotted against dose in Figure 1b. In the limit of zero dose, G(Mb) = 1.4 ± 0.2 and G(Mb(IV)) = 0.7 ± 0.1. This latter value is fully consistent with H_2O_2 being the dominant source of ferrimyoglobin peroxide production.

The e_{aq}^--induced composition changes were determined in Ar-purged solutions containing 38 ± 2 μM ferriMb and 0.1 *tert*-butyl alcohol in the dose range 1.6–69.7 krad. The corrected composition changes are shown as a function of dose in Figure 2a, where a plateau region is again attained. The solution composition at the steady-state is 64% Mb, 12% Mb(IV), and 24% ferriMb, markedly different from the ·OH analogue. In the limit of zero dose, G(Mb) = 1.6 ± 0.2, as shown in Figure 2b.

In the context of the report by Bernofsky et al (1959), two features of clarification emerge. In their absence of knowledge about attainment of a radiolysis steady-state, these authors did not consider that their aerated product spectrum was a *mixture* of myoglobin derivatives. Both Mb(IV) and MbO_2 may be present, along with unconverted ferriMb. In addition, our results further suggest that the post-irradiation process observed by these authors after room-temperature radiolysis involves the ferri-peroxide and deoxy forms of myoglobin as reactants. Further details of this process will be reported elsewhere.

A N_2O-saturated 27.5 μM ferriMb solution γ-irradiated to 14.9 krad was thoroughly aerated after following the deaerated post-irradiation process for 6 min. Upon aeration, the Mb product quantitatively converts to MbO_2 which then continues the thermal reaction at a similar rate. Composition analysis was based on the MbO_2, ferriMb, and Mb(IV) forms. When N_2O-saturated ferriMb solutions (27.5 μM) are γ-irradiated (3.9–5.5 krad) in the presence of O_2 (0.31–0.46 mM), there is no reductive conversion of ferriMb to MbO_2; the peroxide is the only product with G(Mb(IV)) = 1.3 ± 0.1. This compares with G(Mb) ≅ 0.6–0.7 and G(MbIV) ≅ 0.7 (from Figure 1b) in deaerated solution. Those $\cdot O_2^-$ radicals generated from the $\cdot H + O_2 \rightarrow \cdot HO_2$ ($\rightleftharpoons \cdot O_2^- + H^+$) scavenging reaction under the experimental conditions employed would subsequently produce negligible quantities of MbO_2 or ferri-peroxide by reaction with the ferriMb substrate. This assertion follows from the study of Ferradini et al. (1978) on the interaction of $\cdot O_2^-$ with ferrihemoglobin.

These quantitative results clarify the Giddings and Markakis (1972) report that, depending on solution deaeration, MbO_2 and Mb(IV) are separately generated in ferriMb radiolysis. That G(Mb(IV)) obtained in the presence of O_2 is similar to G(Mb) + G(Mb(IV)) obtained by radiolysis in the absence of O_2 suggests that the extra peroxide generated in its presence, beyond that accountable by reaction of H_2O_2, may result from a diversion of intermediate reactivity away from reductive conversion toward peroxide production. We find no support for the claim by these

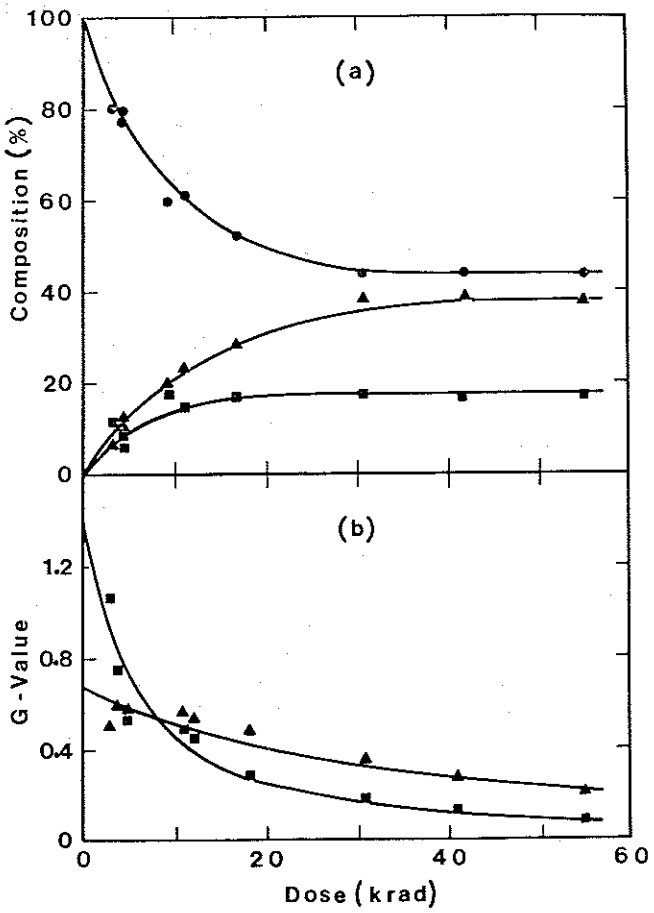

Fig. 1—(a) γ-induced composition changes; (b) G-values, as functions of dose for N_2O-saturated solutions, 30 ± 2 μM in myoglobin at pH 7.3 Ferrimyoglobin (●), deoxymyoglobin (■), ferrimyglobin peroxide (▲).

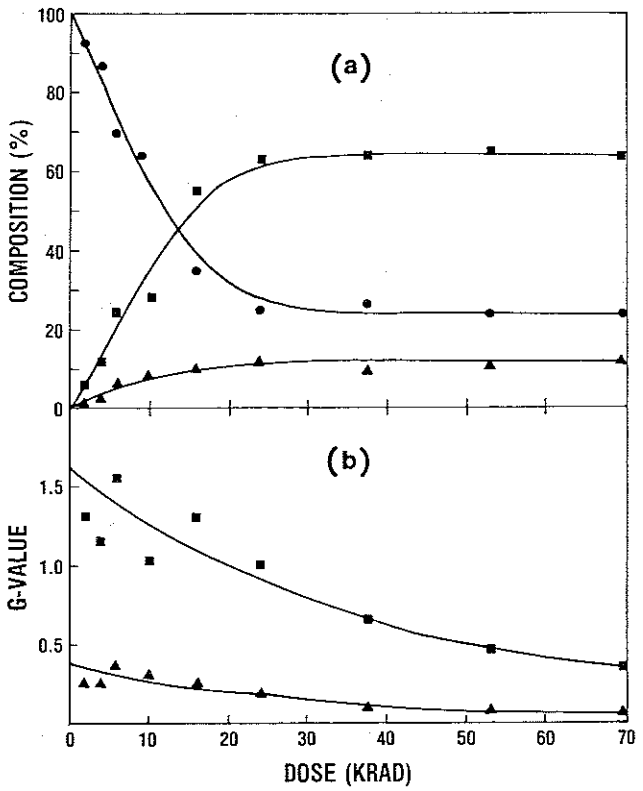

Fig. 2—(a) γ-induced composition changes; (b) G-values, as functions of dose for Ar-purged solutions, 38 ± 2 μM in myoglobin and 0.1M in tert-butyl alcohol at pH 7.3. Ferrimyoglobin (●), deoxymyoglobin (■), ferrimyoglobin peroxide (▲).

authors that MbO_2 is solely generated in deaerated solution; our quantitative analysis clearly demonstrates only Mb and Mb(IV) production. Since H_2O_2 radiolysis-generation is independent of aeration, and the ferriMb + H_2O_2 → ferri-peroxide reaction evolves O_2 rather than requiring it (King and Winfield, 1963), we assert that the peroxide must also be generated under deaerated conditions. In addition, arguments presented by these authors for O_2 production via $\cdot HO_2$ radical involvement have no basis, since this radical is not a radiolysis product in deaerated solution. The "residual oxygen" they have reported as a possible source of Mb → MbO_2 conversion might possibly result from air-leakage into their solution vessel during "irradiation under vacuum."

The novel features that have emerged in this study have enabled us to correct earlier misconceptions about the nature of the radiolysis of ferri-myoglobin in aqueous solution. As a consequence, a better perspective is provided of the radiation-induced color changes in meat samples. The radiation dose delivered and the time of product analysis after irradiation must be considered when radiolysis of meat samples or extracts are compared with regard to color change phenomena.

REFERENCES

Anbar, M., Bambaneck, M., and Ross, A.B. 1972. "Selected Specific Rates of Reactions of Transients from Water in Aqueous Solution. 1. Hydrated Electron." Natl. Stand. Ref. Data Ser., Natl. Bur. Stand., No. 43.

Bernofsky, C., Fox, J.B., and Schweigert, B.S. 1959. The effects of low dosage gamma irradiation on beef myoglobin. Arch. Biochem. Biophys. 80: 9.

Brown, W.D. and Akoyunoglou, J.H.A. 1964. γ-irradiation of purified myoglobins. Arch. Biochem. Biophys. 107: 239.

Clarke, R. and Richards, J.F. 1971. Effect of γ-irradiation on beef myoglobin. J. Agr. Food Chem. 19: 170.

Ferradini, C., Foos, J., Gilles, L., Haristoy, D., and Pucheault, J. 1978. Gamma and pulse radiolysis studies of the reaction between superoxide ions and oxyhemoglobin-methemoglobin system. Photochem. Photobiol. 28: 851.

Fox, J.B., Nicholas, R.A., Ackerman, S.A., and Swift, C.E. 1974. A multiple wavelength analysis of the reaction between hydrogen peroxide and metmyoglobin. Biochemistry 13: 5178.

Giddings, G.G. and Markakis, P. 1972. Characterization of the red pigments produced from ferrimyoglobin by ionizing radiation. J. Food Sci. 37: 361.

Hardman, K.D., Eylar, E.H., Ray, D.K., Banaszak, L.J., and Gurd, F.R.N. 1966. Isolation of sperm whale myoglobin by low temperature fractionation with ethanol and metallic ions. J. Biol. Chem. 241: 432.

King, N.K. and Winfield, M.E. 1963. The mechanism of metmyoglobin oxidation. J. Biol. Chem. 238: 1520.

Satterlee, L.D., Brown, W.D., and Lycometros, C. 1972. Stability and characteristics of the pigment produced by gamma irradiation of metmyoglobin. J. Food Sci. 37: 213.

Shieh, J.J., Sellers, R.M., Hoffman, M.Z., and Taub, I.A. 1979. Radiation chemistry of myoglobin, its derivatives, and model compounds in aqueous solutions. In "Radiation Biology and Chemistry: Research Developments," Ed. Edwards, H.E., Navaratram, S., Parsons, B.J., and Phillips, G.O., p. 179. Elsevier, Amsterdam.

Tappel, A.L. 1956. Regeneration and stability of oxymyoglobin in some gamma irradiated meats. Food Res. 21: 650.

Yamazaki, I., Yokota, K., and Shikama, K. 1964. Preparation of crystalline oxymyoglobin from horse heart. J. Biol. Chem. 239: 4151.

Ms received 2/14/81; revised 6/11/81; accepted 6/15/81.

The preparation of the myoglobin extracts by Dr. J.J. Shieh is gratefully acknowledged. This research was supported by US Army Contract DAAG60-79-C0005 to Boston University.